WHO Advisory Committee on Variola Virus Research

Report of the Nineteenth Meeting

Geneva, Switzerland

1 and 2 November 2017
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Executive summary

The Advisory Committee on Variola Virus Research held its nineteenth meeting in Geneva on 1 and 2 November 2017. It considered the implications of its discussions in light of the upcoming substantive agenda item at the Seventy-second World Health Assembly in May 2019 on the destruction of variola virus stocks. Only one year remained for the Advisory Committee to draw conclusions from the outcomes of on-going research which would form the basis of its unequivocal advice to the Director-General about the current status of variola virus research.

WHO Smallpox Secretariat

The WHO Smallpox Secretariat outlined the reporting process to WHO’s governing bodies over the next two years and the implications for the Advisory Committee’s work. It also reported on recent developments including outbreaks of monkeypox, an emerging orthopoxvirus, in Africa. The link between variola virus research work and the prevention and control of monkeypox was noted and appreciated.

An update was presented on WHO’s Smallpox Vaccine Emergency Stockpile and the Smallpox Vaccine Operational Framework which would be activated in the event of a smallpox event. WHO’s work on the use of experimental vaccines and assessment of smallpox vaccines was outlined.

WHO’s biosafety inspection team completed the current cycle of visits to the two authorized repositories of variola virus: the State Research Centre for Virology and Biotechnology (VECTOR), Koltsovo, Novosibirsk Region, Russian Federation in October 2016 and the US Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, United States of America (USA) in May 2017. The reports have been finalized and are available on the page of the WHO Smallpox Secretariat on the WHO website.

Reports on the collections of variola virus

The Advisory Committee received reports on the virus collections held at the two repositories, both WHO Collaborating Centres. In the past year, no withdrawals or additions had been made to either collection, and no genetic material had been transferred to other laboratories.

Update on research proposals

Six proposals for continuing research using live variola virus had been received from the two Collaborating Centres in 2017 (five from CDC and one from VECTOR), of which five had been (all from CDC) accepted as being of essential public health benefit.

Update on on-going research projects

The Advisory Committee was updated on progress in approved, continuing research projects using live variola virus for the development of diagnostic tests, animal models, smallpox vaccines, and antiviral and therapeutic agents, on work in the private sector on smallpox vaccine and antiviral agents, and regulatory perspectives.

Participants from CDC reported to the Advisory Committee on advances in protein-based diagnostic tests, which offered good prospects for the development of point-of-care tests. Work included preparation and use of human monoclonal antibodies, variola virus-specific antigen capture assays, lateral flow assays and protein microarrays. CDC’s advisors also described work on real-time PCR-based diagnostics. One such assay was licensed by the Food and Drug Administration (FDA) in the USA in early 2017. Further work is focusing on an assay to detect all known pathogenic human orthopoxviruses. CDC also reported results of work on humanized mice as a model for smallpox and on the use of live variola virus in developing less-reactogenic, third-generation smallpox vaccines. The need for a better understanding of humoral responses and correlates of infection was identified.

Participants from VECTOR described progress in developing a vaccine from a recombinant strain of vaccinia virus that is less reactogenic and neurovirulent as well as more immunogenic and protective.
than the vaccine derived from the parent strain. Preclinical studies are in progress. Researchers at VECTOR also used viral sequencing to establish the phylogeny of strains of variola virus isolated from patients during an outbreak of smallpox in Moscow in 1960, showing the high stability of the virus during the outbreak.

**Update on progress towards licensure of antivirals and vaccines**

The manufacturer of tecovirimat informed the Advisory Committee that all the necessary studies for regulatory approval of its oral formulation had been completed and submission of an application was in preparation, with a decision hoped for in the third quarter of 2018. The company was working with FDA towards submission for regulatory approval of an intravenous formulation.

The Advisory Committee was also updated about the development of brincidofovir, a second antiviral agent being considered as a treatment for smallpox but which is also used to treat patients infected with other double-stranded DNA viruses such as cytomegalovirus and adenovirus. Those studies have shown that the antiviral has acceptable safety and tolerability.

The manufacturer of the non-replicating MVA-based smallpox vaccine Imvamune®/Imvanex® reported that regulatory studies in preparation for an application for FDA approval were under way. The aim was to submit an application in the second half of 2018.

The Advisory Committee was updated on FDA’s perspectives on the development and approval of smallpox countermeasures, including the evolution of its Animal Rule and the giving of Pre-Emergency Use Authorization for Imvamune®.

**Recommendations and observations**

**Diagnostics**

The Advisory Committee welcomed FDA’s licensing of a new variola virus-specific, real-time PCR diagnostic test, and its availability to laboratories that are members of the US Laboratory Response Network. It also welcomed the considerable progress that has been made in the development of protein-based diagnostic tests and the prospects for their use in the field. Members of the Advisory Committee were divided on whether the use of live variola virus for their further development of diagnostics would be considered as “essential for public health”.

**Antiviral agents**

The Advisory Committee appreciated the updates on potential therapeutic agents against smallpox, tecovirimat and brincidofovir, and welcomed the likely imminent submission of an application for licensure of tecovirimat to FDA. It noted that at least 24 months’ more work was needed before an application for licensure of brincidofovir for use against variola virus was likely. It was encouraged by early results on the use of a combination of human monoclonal antibodies to neutralize variola virus.

**Smallpox vaccines**

The Advisory Committee further welcomed the information that an application for approval of a highly-attenuated, MVA-based smallpox vaccine was expected to be submitted in 2018 to FDA for licensure in the USA.

**Scientific Subcommittee**

The Advisory Committee decided that, instead of using a Scientific Subcommittee, in future the full Advisory Committee would evaluate proposals submitted by the two WHO Collaborating Centres for on-going research involving the use of live variola virus. The WHO Secretariat would administer the process.
1. Opening of the meeting

The Advisory Committee on Variola Virus Research held its nineteenth meeting, in Geneva, on 1 and 2 November under the chairmanship of Professor G.L. Smith with Mr D.W. FitzSimons as Rapporteur.

The Advisory Committee adopted its agenda.

Staff members of WHO’s Office of the Legal Counsel advised the meeting that WHO’s constitutional mandate included the need to ensure impartiality and independence in all advice that it receives from advisory groups such as the Advisory Committee on Variola Virus Research. They described the procedure for evaluation by the WHO Secretariat of participants’ potential or perceived conflicts of interest (including financial, personal and professional) before their attendance, based on their declaration of conflicts of interest forms.

Dr S. Briand, Director of the Department of Infectious Hazard Management within the WHO Health Emergencies Programme, opened the meeting. She thanked the Committee members for their commitment and long-standing service in overseeing the research on vaccines, diagnostics and antivirals for smallpox since 1999.

WHO’s new Director-General, appointed in May 2017, was committed to change, including a greater role for working in partnership and the strengthening of countries’ capacities in their responses to outbreaks of disease and the reducing of risk. Dr Briand recounted a recent breach of airport security by a seven-year-old girl, which illustrated the fact that no matter how fool-proof procedures may appear to be there was always a risk. In the case of smallpox, even a minimal risk had to be prepared for and the Advisory Committee’s work lay at the heart of global health security.

Dr Briand outlined the process of reporting on the Advisory Committee’s conclusions to WHO’s governing bodies, which would culminate in the submission of a substantive report to the Executive Board at its 144th session in January 2019 and subsequent debate in the Seventy-second World Health Assembly in May 2019 on the destruction of variola virus stocks. She recalled that the Advisory Committee in its previous meetings had recommended
that live variola virus was needed only in research on antiviral agents. For that reason, the agenda of the present meeting included updates on progress towards licensure of some candidate compounds. The Sixty-ninth World Health Assembly in 2016 agreed to defer its decision for three years until the Seventy-second World Health Assembly in 2019 in order to allow completion of agreed research that should provide the basis for a more conclusive discussion.

The role of the Advisory Committee is to provide impartial, independent advice to inform the decision-making process on the date of destruction of variola virus stocks. The pool of expertise on variola virus and smallpox was small and the membership of the Advisory Committee had been expanded to bring in more experts on public health and synthetic biology, while ensuring high-quality input in line with the WHO mandate to the Advisory Committee.

The Advisory Committee members and advisors introduced themselves.

2. Report of the WHO Secretariat - Dr A. Khalakdina

Dr Khalakdina outlined the process of reporting to WHO’s governing bodies since the adoption of resolution WHA49.10 in 1996 on the destruction of the remaining stocks of variola virus. The Seventieth World Health Assembly in May 2017 had noted the progress report on destruction of variola virus stocks and approved the inclusion of the subject as a substantive item on the provisional agenda of the Seventy-second World Health Assembly in May 2019. In the interim, a report of the Advisory Committee’s present meeting would be submitted to the Seventy-first World Health Assembly in May 2018. The Advisory Committee would meet again in October 2018 and its conclusions would be submitted to the Executive Board at its 144th session in January 2019.

The annual progress reports to the governing bodies, in all WHO’s official languages, and the governing bodies’ discussion as well as the reports of the Advisory Committee and the reports on the inspection visits of the two collaborating centres were all in the public domain and accessible on the WHO website.
The WHO Secretariat had reviewed the events surrounding the synthesis of horsepox virus reported to the Advisory Committee at its eighteenth meeting, and after receiving advice from WHO Legal Counsel decided that the links of one of its members with the private sector sponsoring this work were not in the Organization’s best interests. Several members of the Advisory Committee expressed their disappointment with the decision especially in view of the significant contribution that this member had made to the Committee’s activities over a number of years.

She briefly outlined the situation regarding monkeypox. Cases had been reported from the Central African Republic, Congo, Democratic Republic of the Congo, and most recently Nigeria (where no case had been reported since the 1970s). The Secretariat was working with Member States to improve laboratory capacity, improve risk communication and engage other partners and networks. The detection of outbreaks illustrated that surveillance was effective, but it was evident that better coordination for control was needed. As the issues were relevant to about the work on smallpox and its vaccines, the Secretariat was convening an informal consultation immediately after the Advisory Committee’s meeting (Geneva, 3 November 2017). Its aim was to ensure that countries at-risk had better capacities to conduct surveillance for and diagnosis of orthopoxvirus infections and to prevent and manage outbreaks of monkeypox.

In discussion it was asked whether, because smallpox vaccines cross-protect against other orthopoxviruses, consideration should be given to using vaccination against variola virus to provide protection against monkeypox. CDC was currently conducting a study to determine the potential protection provided by Imvamune® in health care workers in the Democratic Republic of the Congo.

3. Update on research proposals submitted to WHO in 2017 – Dr G. McFadden

The Advisory Committee was updated on continuing research projects using live variola virus for the development of diagnostic tests, animal models, smallpox vaccines, and antiviral and therapeutic agents. These projects had been previously approved by WHO based on the recommendations of the Advisory Committee through its Scientific Subcommittee.
The Scientific Subcommittee had reviewed six proposals for continuing ongoing research, five from CDC and one from VECTOR (see Annex 1). All those from CDC were accepted. The proposal from VECTOR was rejected, but with suggestions for revision before resubmission.

The CDC proposals covered: use of live variola virus to evaluate antivirals against variola; use of live variola virus to determine whether mice are a suitable animal model for human smallpox; use of live variola virus to develop protein-based diagnostic and detection assays specific for variola virus; use of live variola virus to maintain and regenerate non-infectious variola-derived materials for diagnostic development support; and use of live variola virus to support less-reactogenic vaccine development. The VECTOR proposal was for the development of animal models to study the efficacy of therapeutic and preventive products against smallpox.

The Advisory Committee welcomed the revised process for reviewing the proposals. The scientific justification for completing CDC’s project 2, with a mouse model, was evident, but its public health value was questioned. The Subcommittee had addressed that issue and opinions were divided. It agreed that substantiating initial findings was important to ensure that the Advisory Committee could rely on the scientific conclusions for its advice and recommendations. Moreover, without confirmed conclusions the data could be lost from the permanent record.

4. Update on the Smallpox Vaccine Emergency Stockpile and Smallpox Vaccine Operational Framework – Mr T. Nguyen

WHO’s Smallpox Vaccine Emergency Stockpile currently comprises about 34 million doses of smallpox vaccine. A physical stockpile of approximately 2.4 million doses is held in Switzerland and the rest are held in donor countries. WHO, with the support of the Global Health Security Initiative, has recently developed an operational framework for deploying smallpox vaccine from the emergency stockpile. The framework outlines the processes of
donation and release of appropriate vaccines in response to a smallpox event.\(^1\) Criteria for release of vaccine have been established that satisfy the conditions of the International Health Regulations (2005) and the Strategic Advisory Group of Experts on immunization; the criteria require a comprehensive plan of action for deployment.

In order for WHO to ensure that donated vaccines meet its criteria for inclusion in the Smallpox Vaccine Emergency Stockpile, WHO reviews data on safety, immunogenicity and efficacy before accepting vaccines. The Secretariat is working with potential recipient countries to increase regulatory preparedness and build the capacity of their national regulatory authorities to allow emergency import and to assess medical countermeasures during public health emergencies.

The Advisory Committee recognized the difficulties of several of these issues, such as governance and the potentially long time scale for processing a request.

5. **Reports on the variola virus collection at the WHO Collaborating Center for Orthopoxvirus Diagnosis and Repository for Variola Virus Strains and DNA at VECTOR, Koltsvo, Novosibirsk Region, Russian Federation – Dr S.N. Shchelkunov**

Dr Shchelkunov reported that the organization of and research with live variola virus at VECTOR complied with international and the Russian Federation’s requirements as well as WHO’s recommendations. The collection contains 120 strains originating in countries from all WHO regions except that of the Western Pacific, including 49 from Africa and 34 from South-east Asia.

The conditions of storage of the virus in the repository were rigorous and secure, satisfying the applicable national regulations. Freezers had alarm systems and operating parameters were continuously displayed for monitoring. Electrical supplies include back-ups and independent generators. Access was constantly monitored, strictly limited and regulated. For both inventory checking and research, air-tight metal containers with variola virus were

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moved into BSL-4 laboratories located in the same building as the variola virus repository facility. The WHO biosafety inspection team confirmed during its visit in October 2016 that the variola virus storage conditions complied with current international requirements.

No research with live variola virus had been undertaken so far in 2017; a federal certificate permitting such work was re-issued in August 2017. Research with live variola virus (to first be approved by WHO) which was planned for the next 14 months, covered discovery of new therapeutic and preventive antivirals, and assessment of the neutralizing activity of vaccinees’ blood sera.

6. Reports on the variola virus collection at the WHO Collaborating Center for Smallpox and other Poxviruses at the Centers for Disease Control and Prevention, Atlanta, Georgia, USA – Dr I. Damon

Dr Damon reported the status of the collection. No withdrawals or additions were made to the two long-term repositories. The laboratory working stocks were inventoried in April 2017. Between October 2016 and October 2017, 30 removals (involving two strains) were made for use in approved research projects. In the same period, no transfer of genomic material was made to other laboratories.

The high-containment laboratory was in active use until April 2017 when it was shut down and decontaminated. Preventive maintenance was undertaken in April 2017 and the facility was inspected by the WHO biosafety team in May 2017. Operations on the five research projects were resumed in June 2017.

In 2017, methods to sequence viruses were optimized, enabling direct sequencing of viruses from scab material without propagation. Multiplex whole-genome sequencing was performed on 24 samples of variola virus, and phylogenetic tree clusters were established for 22 of them.

Dr Damon also described early work with a nanopore sequencing system for rapid, deployable identification of poxviruses. Procedural enhancements and next-generation sequencing platforms offer good prospects for field diagnostic or epidemiological tools.
7. Use of live variola virus to develop protein-based diagnostics and detection assays specific for variola virus – Dr V. Olson

A goal of the research on diagnostic assays is to identify those that are well suited for use in resource-constrained settings. Dr Olson described developments in protein-based diagnostics including preparation and characterization of human monoclonal antibodies, variola virus-specific virus capture assays, the potential of immunofiltration or lateral flow assays, and a chip diagnostic with a variola virus-encoded protein microarray.

Highly specific monoclonal antibodies (mainly IgG) have been generated against unique protein targets. Screening narrowed these targets down to six proteins on the surface of intracellular mature virus and extracellular enveloped virus and a virulence factor. The antibodies were used in viral antigen-capture assays and demonstrated a concentration-dependent capture response to viral antigen. The monoclonal antibodies had differential capture abilities to live virus versus γ-irradiated virus, with some unable to capture the live virus agent. Initial tests in a lateral flow assay gave promising results.

Column-based immunofiltration assays are rapid, sensitive, easy to deploy, user-friendly and can be adapted as point-of-care tests for all orthopoxviruses, with a good limit of detection for five different orthopoxviruses. One assay successfully identified monkeypox virus antigen in clinical samples from suspected cases and shows promise for the development with variola virus-specific monoclonal antibodies as a rapid detection assay for use in resource-constrained settings.

For a variola proteome microarray, a chip has been developed with 189 unique variola virus-encoded proteins. The concept that it could be useful in differentiating the antibody response of vaccinees from that of people who have had an exposure to a systemic orthopoxvirus infection was validated in tests with sera from people potentially exposed to the Akhmeta orthopoxvirus. The chip appears to be able to differentiate vaccination (vaccinia virus) from other orthopoxvirus infections.

In discussion, the scientific merit and potential of the work were recognized, but questions were raised about the use of live virus for development of diagnostics and the time needed to
complete the work. If variola virus were destroyed in 2019, consideration should be given to preparing stocks of γ-irradiated virus in advance.

It was pointed out that earlier research had focused on real-time PCR, but the recent developments in protein-based diagnostics opened the way to public-private partnerships for developing point-of-care tests. One member of the Advisory Committee asked whether protein-based assays would be used in the front line or when an outbreak had been confirmed. Their stability would obviate transfer of samples to laboratories, which would have clear advantages.

8. **Use of live variola virus to evaluate antiviral agents against smallpox** – Dr V. Olson

Dr Olson described collaborative work with mixtures of human monoclonal antibodies against different orthopoxviruses. Two mixtures neutralized vaccinia virus (both intracellular mature virus and extracellular enveloped virus) more effectively than vaccinia immune globulin. In mice challenged with vaccinia virus, Mix6, fully protected all challenged animals.

As the mouse model has its limitations, researchers turned to monkeypox virus infection in prairie dogs, in which disease progression is closer to that seen in humans. The animals tolerated the monoclonal antibodies well and the longevity of the antibodies within the model is currently being determined using a neutralization assay. Commercial entities have also developed monoclonal antibodies against orthopoxviruses. All the monoclonal antibodies developed neutralized monkeypox virus in vitro better than vaccinia immune globulin and were stable for long periods. One cocktail shows promising results against different orthopoxviruses (vaccinia, ectromelia and monkeypox viruses) in several animal models (mice and non-human primates), again with better protection than with vaccinia immune globulin. The cocktail was more effective than its components individually. In macaques the monoclonal antibodies showed a half-life of more than one week; survival declined to 80% when treatment was administered in the second week after challenge from 100% when treatment was administered on days 1 and 7 after challenge. Quantitative PCR of collected tissues showed lowered levels of viral nucleic acid in all tissues in treated animals, and in
some instances no viral DNA was detected. Morbidity was also greatly reduced, even when the antibodies were given 6 days after infection.

9. Use of live variola virus to determine whether mice are a suitable animal model for human smallpox – Dr C. Hutson

Dr Hutson recalled that a good animal model of human smallpox has been elusive. An ideal animal model would: have a route of infection that mimics the natural transmission of the pathogen; induce disease with an infectious dose equivalent to that causing disease in humans; and have a disease course, morbidity and mortality similar to that seen in human disease. Accordingly, humanized mice are being studied; these animals are “created” on the NOD scid gamma (NSG) background mouse and are “humanized” through the use of human CD34$^+$ cells, human peripheral blood mononuclear cells (PBMCs) and/or human tissue engraftments. Variola virus can replicate and cause morbidity and mortality in these animals.

In discussion, several members of the Advisory Committee questioned the feasibility and applicability of the model and whether its use was essential for public health research.

10. Use of live variola virus to support less-reactogenic vaccine development: continued evaluation of “third” generation vaccines – Dr C. Hutson

Dr Hutson presented the findings of studies done in collaboration with Bavarian Nordic on Imvamune® and with the National Institute of Infectious Disease in Tokyo, Japan, on LC16m8 vaccine. Both sets of studies focused on understanding the ability of sera from subjects vaccinated with third-generation vaccines (Imvamune® or LC16m8) to neutralize variola virus. A better understanding is needed of the humoral responses and the correlates of protection with newer vaccines (LC16m8 and Imvamune®).

The collaboration with Bavarian Nordic examined the ability of sera from people in a phase III trial of Imvamune® versus ACAM2000 (a non-inferiority study) to neutralize variola virus. The existing plaque-reduction neutralization test (PRNT) for intracellular mature variola virus was adapted to resemble more closely the Bavarian Nordic PRNT (which
established the sera’s ability to neutralize vaccinia virus), and standard operating procedures were harmonized and validated. The assay was modified slightly to achieve about 100 plaques per well and incorporated a macro-analyzer for quality control and consistency. Re-evaluation of CDC’s assay confirmed reproducibility. The sensitivity of the assay in distinguishing between different groups of sera (based on neutralization) was also confirmed. The testing demonstrated statistically significant differences between high-, medium- and low-PRNT responder sera. The adapted assay was also shown to be more robust and less subjective, allowing for good quality control. It gave results that were reproducible between operators and days. Procedures for use of the assay have been developed. So far, results show that both LC16m8 and Dryvax caused a rise in neutralizing titre, the extent depending on the neutralization antigen.

In discussion, it was noted that neutralization does not necessarily correlate with protection and the correlates of protection remain unknown.

11. Use of live variola virus to maintain and generate non-infectious variola derived materials for diagnostic development support – Dr V. Olson

A core function of the WHO Collaborating Center on Smallpox is to validate diagnostic assays designed for specific detection of variola virus should a variola-like virus emerge. Dr Olson described the considerable advances that have been made through work on real-time PCR DNA-based diagnostics. An application to the US Food and Drug Administration (FDA) for regulatory approval of a variola virus species-specific diagnostic assay for use within the Laboratory Response Network in the USA was submitted in 2016 and approved in February 2017.

The assay uses primers and probes for two variola virus genes and was validated with three strains of variola virus and other related orthopoxviruses and 20 other pathogens causing rashes or found on the skin. The limit of detection was 50 fg of genomic DNA. The findings were reproducible between operators and over time. Work is under way to convert the assay into a multiplex format to include the variola virus specific tests, a generic orthopoxvirus assay, and a sample integrity control. The aim is to create a real-time PCR assay that can detect variola virus, with the proper controls, in a single reaction. The assay is being
optimized for use with a platform that is currently being used for rapid detection of monkeypox virus in clinical samples from the Democratic Republic of the Congo. Initial results are encouraging.

Through a public-private partnership another assay was being developed to detect all known pathogenic human orthopoxviruses and differentiate variola virus. The assay showed good specificity and had a low limit of detection of cowpox and variola viruses. Multiple strains of variola virus were reproducibly detected by the variola virus-specific probe. The specificity of the assay was retained in the presence of Akhmeta virus and the Alaska isolate, and tests were under way to determine that the presence of other orthopoxviruses did not affect the sensitivity of the assay to detect variola virus.

Work continued to validate new variola virus-specific assays that target different regions of the viral genome. A tool kit with multiple diagnostic assays would provide better preparedness for detection should a variola-like virus emerge.

The Advisory Committee welcomed the news of FDA’s approval as a major advance, although it emphasized that it was available for defined laboratories and not commercially. In response to a question about the comparative sensitivity between this DNA-based assay and the protein-based assays discussed earlier in the meeting, it was observed that there was only one order of magnitude difference: the FDA-approved DNA diagnostic assay could detect about 50 000 genomes/ml whereas the ABICAP protein-based assay was less sensitive with a detection level of 500 000 genomes/ml.

12. Preclinical studies on a candidate smallpox vaccine, VACdelta6 – Dr S.N. Shchelkunov

Based on the vaccinia virus strain LIVP that is used for human vaccinations in the Russian Federation, a recombinant variant of the virus, VACΔ6, has been designed. Researchers made directed alterations of the genes encoding γ-interferon-binding protein (B8R), complement-binding protein (C3L), haemagglutinin (A56R), thymidine kinase (J2R), the Bcl2-like inhibitor of apoptosis (N1L), and the A35R gene that controls antigen presentation by major histocompatibility complex (class II) molecules. The A35R gene was deleted to enhance
immunogenicity on the basis that the result had been observed for the modified vaccinia Ankara (MVA) strain of the virus.

An efficacy study of LIVP and VACΔ6 in CV-1, Vero and 4647 cell cultures did not reveal any significant differences in this parameter between the viruses compared.

Three batches of a candidate vaccine against smallpox based on VACΔ6 have been produced and preclinical studies on the specific activity of this vaccine in vitro and in vivo have been conducted. Vaccination with VACΔ6 leads to reduced reactogenicity and neurovirulence compared with the parent LIVP. VACΔ6 is more immunogenic and protective than the parent LIVP strain.

In discussion, Dr Shchelnukov explained that the preclinical studies were due to end in 2018, whereupon clinical research would begin.

13. Retrospective genetic analysis of genomes of variola virus strains – Dr E.V. Gavriloava

Dr Gavriloava described the results of a genetic analysis of variola virus isolated during an outbreak of smallpox in Moscow in 1960 following the return of a patient from a two-week duty travel to India. In the resulting outbreak, 47 people were infected and 8 died (a mortality rate of 8.5%). Strains of virus were isolated from four patients and the complete genomes were sequenced. The average sequencing coverage varied for the four strains. Phylogenetic analysis placed all the strains on the same branch of the phylogenetic tree, close to isolates from Bangladesh and Nepal. Only minor differences were seen between the four genomes, mostly in the number of AT repeats (10-14) in one region. The data show the high stability of the virus during the outbreak.

In separate research, the full sequences of 24 strains of variola virus (including four clinical material from crusts) from different parts of the world in the Collaborating Centre’s collection were identified. Four isolates from two separate outbreaks in Tanzania in 1962 were analysed and showed up to 138-146 single nucleotide polymorphisms.
The newly defined sequences were combined with 49 sequences of variola virus present in GenBank to generate a phylogenetic tree.

In discussion, it was observed that CDC have just finished sequencing another 22 genomes. That brings the total known to at least nearly 100, and more may be about to be published. It was hoped that all the new sequences would be posted in GenBank.

14. Update on smallpox vaccine IMVANEX® (IMVAMUNE®) – Dr N. Samy

Dr Samy outlined the product profile of her company’s non-replicating MVA-based vaccine, licensed (as Imvanex®) in the European Union and, for limited use, in Canada (as Imvamune®). It has been granted a pre-emergency use authorization status in the USA where Imvamune® is currently stockpiled by the US Government for emergency use in people for whom replicating smallpox vaccines are contraindicated. It is available as a sterile, liquid frozen, live attenuated poxvirus suspension for subcutaneous administration in two doses (for immunization of vaccinia-naive subjects) or as a single dose (for boosting the immune response in vaccinia-experienced subjects). Its shelf-life extends currently to nine years at -80 °C. It is well tolerated and has generated no safety concerns in more than 9000 vaccinated subjects (altogether having received more than 16 000 doses) including people who are HIV-infected or who have atopic dermatitis; there is no evidence of cardiac risk. The safety profile in at-risk populations (HIV-infected subjects and people with atopic dermatitis) is comparable to that in healthy individuals. It is aimed at both first-line responders and the whole general population.

Altogether 19 clinical trials have been completed. The results of a non-inferiority trial with ACAM 2000 and additional data on HIV-infected subjects are being analysed. The vaccine generates comparable immune responses to Dryvax in humans, and even a single dose induces the same long-lived memory as two doses.

In a collaborative observational study of the vaccine with CDC in some 1000 health care workers at risk of monkeypox virus infection in the Democratic Republic of the Congo no serious adverse event or monkeypox virus infection has been seen.
In discussion, Dr Samy emphasized that the company had a high manufacturing capacity and had already delivered some 28 million doses of Imvamune® to the US Strategic National Stockpile. The company intends to file a Biologics License Application with FDA by the second half of 2018 and is expecting a decision in 2019.

No specific studies have been performed with Imvamune® in pregnant women, but about 30 pregnancies have been reported so far during the clinical development of the vaccine. In the monkeypox study, although there had been no plans to include pregnant women, 20 women were found to be pregnant. Collectively, the pregnancy outcomes were overall comparable to the experience in the general population.

Regarding durability of immune responses, antibodies have declined at the two-year follow-up, but all subjects tested showed a strong boost response to a single booster dose, indicating a robust memory response.

15. Progress towards approval and deployment of TPOXX® (ST-246) - Dr D. Hruby

Dr Hruby reported the status of the development of both oral and intravenous formulations of tecovirimat (ST-246, TPOXX®) for the treatment of smallpox and other human orthopoxvirus infections. For the oral formulation of tecovirimat all the necessary studies have been completed and the New Drug Application is being provided to FDA as a rolling submission with the final modules expected to be filed by the end of 2017. If satisfactory, regulatory approval is expected in the third quarter of 2018.

For intravenous administration of tecovirimat the company has completed a single ascending-dose clinical trial. No severe adverse events were noted, and dose linearity was observed, which allows an appropriate intravenous dose to be selected to approximate the pharmacokinetic parameters measured with following oral administration of tecovirimat. Following recent FDA guidance, the company is moving forward with a bioavailability/multiple dose study to advance its programme towards a subsequent regulatory filing for the intravenous tecovirimat product. It is evaluating palatability for potential paediatric recipients by crushing and mixing the product with milk and apple sauce in order to enable weight-based dosing for infants and potentially geriatric populations. Work
includes human factor studies to examine how individuals follow instructions for the proper mixing into foods.

The manufacturer has completed delivery of the initial order of two million courses of tecovirimat to the US Strategic National Stockpile.

16. **Update on the development of brincidofovir (CMX001) for smallpox – Dr F. Gray**

Dr Gray summarized progress in the development of brincidofovir as an antiviral against numerous double-stranded DNA viruses including variola virus. It has been administered to more than 1400 patients with cytomegalovirus or adenovirus infections in open-label and named patient/emergency programmes including immunosuppressed subjects, people with renal and/or hepatic impairments and paediatric patients as young as one month of age. In initial studies in patients with severe adenovirus infection early treatment is associated with improved survival rates.

Several factors support use of brincidofovir in smallpox. It shows potent antiviral activity in vitro and consistent and reproducible efficacy in animal models (mouse/ectromelia virus and rabbit/rabbitpox virus). It is noteworthy that generation of resistance in orthopoxviruses in vitro is typically slow (months), requires multiple mutations for high-level resistance, and resistant strains have impaired growth, suggesting a high barrier to resistance to brincidofovir. Furthermore, there is an extensive clinical safety database from multiple studies against double-stranded DNA viruses. The manufacturer has advanced formulation experience and large manufacturing capacity and readiness. Brincidofovir can be administered intravenously and orally and can be offered in blister packs. Overall it offers multiple desirable characteristics for use in a public health emergency.

The company continues to work in partnership with the Biomedical Advanced Research and Development Authority (BARDA) and FDA in the USA to develop brincidofovir for use against smallpox. In 2016 the European Commission granted orphan designation to the company for the use of brincidofovir to treat smallpox. The company is seeking scientific
advice for the development of brincidofovir for use against smallpox from the European Medicine Agency’s Committee for Medicinal Products for Human Use.

It is expected that human dosing for smallpox will be 200 mg/week for three weeks. At that dose regimen in humans, brincidofovir was generally well tolerated in both adults and paediatric patients, with the most common adverse events being diarrhoea and other gastrointestinal disturbances.

In discussion, it was noted that in one case of human cowpox and one of vaccinia brincidofovir in combination with other anti-orthopoxvirus agents was effective.

17. FDA perspectives on the development and approval of smallpox medical countermeasures – Dr E. Cox

Dr Cox outlined the inventory of diagnostics, treatments and vaccines available as medical countermeasures against smallpox. For diagnosis, FDA has approved a nucleic acid-based assay to detect non-variola orthopoxviruses. In February 2017 FDA granted a de novo classification request for a real-time PCR assay to detect variola virus.

For antivirals, product development under FDA’s Animal Rule is evolving; FDA’s guidance, published in 2015, indicated that efficacy findings from animal studies may support approval of an antiviral against variola, although the uncertainty in extrapolating from animals to humans always needs consideration. Nevertheless, the Rule does not eliminate the need for human data: safety can be assessed in volunteers and patients with other disease conditions, as is being done with brincidofovir and tecovirimat. Plans are needed for properly designed clinical trials to evaluate whether the antiviral products benefit or harm people, if ethical and feasible. Some medical countermeasures may qualify for FDA’s “priority review” vouchers to expedite the review process.

FDA has given Pre-Emergency Use Authorization for Imvamune®. Its position is that the approach to licensure of next-generation vaccines should be based on the traditional approval pathway, if possible, for example through demonstration of non-inferiority to ACAM2000. One such trial has completed enrolment and work continues on other aspects such as assay
validation and manufacturing issues. A clinical study comparing liquid-frozen and freeze-dried formulations has been completed.

In discussion, Dr Cox noted that FDA receives many data on the use of labelled medicines against other diseases and conditions for which they were licensed. Antiviral agents and smallpox vaccines could be tested against monkeypox in the Democratic Republic of the Congo, provided that certain conditions could be met. That approach, however, strengthened the call for stronger national regulatory authorities and the need for national and international preparedness.

18. Update on variola virus repositories biosafety inspection visits – Dr K. Kojima

Dr Kojima recalled that WHO’s mandate was to maintain biennial inspections of the two repositories in order to establish that the conditions of storage of variola virus and or research met the highest requirements for biosafety and biosecurity, and that the reports on the visits be made public. An international team of prominent experts visited VECTOR in October 2016 and CDC in May 2017, each for one week. The 16-element assessment protocol used was based on the European Committee for Standardization (CEN) Workshop Agreement 15793 (CWA 15793) for continuity and follow-up of previous findings. In each repository a self-assessment form was completed to identify updates and modifications since the previous inspection, so as to provide continuity between inspections.

The inspectors’ conclusions were grouped in four categories: observations – positive remarks or issues not related directly to the scope of the inspection; and priority findings – (a) improvement advisable, (b) timely remedial measures required, and (c) immediate corrective action needed.

The inspectors observed considerable improvements in both repositories, with a substantial reduction in the number of priority findings and closure of most previous findings. Nothing needing immediate corrective action was identified and only a few timely remedial measures were identified. These mostly concerned documentation and training. Both repositories have been asked to draft a workplan for implementing the actions identified.
The reports of the inspections have been finalized, including review by the governments of the countries hosting each of the two repositories for scientific and security redaction. The reports are published on the WHO Smallpox website.

Members of the Advisory Committee expressed concern about the length of time since the visits and the publication of the reports. The Secretariat explained that, for reasons of fairness and equal treatment, the reports were finalized only after the conclusion of the visit to the second repository. Once the report was technically cleared, the respective Member States are given the opportunity for security-related redaction before the report is officially published. At the conclusion of both visits, the findings of the inspection team were communicated to the repository so that necessary action could begin promptly. Dr Kojima emphasized the significant progress seen in both safety and management of the repositories in the past three to four years.

The next cycle of inspection visits is being planned for 2018-2019.

19. General discussion

Preparedness measures for smallpox

1. Antivirals

The prospect of an imminent application for licensure of one candidate anti-variola virus compound was a major step forward. It was observed that the development of one other potential candidate is less far advanced. The Advisory Committee noted and welcomed the potential use of antiviral agents active against variola virus for treatment of monkeypox.

The Advisory Committee discussed the possibilities and regulatory difficulties of repurposing existing antiviral agents active against double-stranded DNA viruses for possible use against orthopoxviruses in emergencies. Different agreements and procedures for their use would be needed. Some existing antivirals could be considered for pre-exposure prophylaxis. Although animal studies with non-variola virus orthopoxviruses showed some positive results, the prospects for pre-exposure prophylaxis being of benefit in clinical cases were not encouraging. In terms of strategy, vaccination would be more effective than treatment.
As with smallpox vaccines and diagnostics, it was difficult to incentivize companies to invest in work on antivirals for a disease that had been eradicated 40 years ago and offered little commercial return. The Advisory Committee recognized this difficulty and applauded the research undertaken in the two collaborating centres. The Committee also noted that brincidofovir offered the prospect of market sustainability because it had other antiviral applications other than orthopoxviruses.

With regard to resistance of variola virus to antivirals, experience from other diseases and use of brincidofovir in an individual with progressive vaccinia pointed to the value of monitoring drug levels and the use of combinations of antiviral agents, although again licensing issues could be an impediment.

2. Diagnostics

**PCR.** The Advisory Committee noted that a real-time PCR test kit had been licensed by FDA in November 2017 for use by specified members of the US Laboratory Response Network. Its provision is accompanied by proficiency training. Criteria for participation in the variola testing capacity of the Network were being reviewed. The WHO African Region had four reference laboratories for viral diseases that could be considered for eligibility for confirmatory diagnostic work on variola virus.

Real-time PCR was useful but had its shortcomings, including difficulties in application in remote settings and proficiency in its use.

**Protein-based diagnostic kits.** Members of the Committee recognized that protein-based diagnostics would be a useful addition to nucleic acid-based (PCR) diagnostic tests, but members were divided between those who argued that such tests were desirable but not essential and those who maintained that their development would make an essential difference. Some members argued that test kits could be evaluated in studies with variola virus proteins produced without use of variola virus, or with \( \gamma \)-irradiated variola virus, for which large quantities could be prepared from live virus before stocks were destroyed.

Members also noted that the pathway to licensure was long and the outcome uncertain, especially in a compressed timescale.
Future research priorities with live variola virus

Given that the report of the Advisory Committee’s next (twentieth) meeting, scheduled to be held in October 2018, will provide an important basis for the discussions of the Seventy-second World Health Assembly in May 2019 on the destruction of variola virus stocks, the Advisory Committee at its present meeting sought to identify what essential research with live variola virus remained to be done.

The Advisory Committee considered the possibility of natural re-emergence of smallpox. As humans were the only reservoir of variola virus and no resurgence had been seen in the 40 years since eradication of the disease, there was little risk of natural re-emergence. However, as it seems likely that variola virus entered humans about 5000 years ago from an animal reservoir, it is not implausible that its precursor survives in some unknown reservoir. What genetic changes in the putative progenitor caused adaptation to humans remain unknown.

The genome of monkeypox virus was the remotest of any known orthopoxvirus from that of variola virus, and mutation into the latter was not likely. Nevertheless, outbreaks of monkeypox in humans were being seen increasingly. Whether this was due to better surveillance, crossing of ecological boundaries, adaptive genetic changes in the virus facilitating human infection, or some combination of these factors was not known. The Advisory Committee noted that many questions remain unanswered.

Other sources of variola virus, apart from unknown stocks, were the two repositories. These, however, were well-organized and the biennial inspections repeatedly instilled confidence in the biosafety and biosecurity measures around these stocks.

Nonetheless, the broadening access to synthetic biology and simplification of the technology are changing the picture. In 2015 the Independent Advisory Group on Public Health Implications of Synthetic Biology Technology Related to Smallpox recognized that variola virus could be recreated with synthetic biology and even modified.¹ The synthesis of horsepox virus, reported to the Advisory Committee at its 18th meeting, proved that an

orthopoxvirus could be synthesized and indicated that this might be of concern to the Health Assembly in 2019 when considering destruction of variola virus stocks.

WHO’s mandate covered preparedness for emergencies, but did not extend to addressing bioterrorism. It maintained a smallpox vaccine stockpile and, in the context of all emerging pathogens, was trying to raise awareness, encouraging the development of medical countermeasures and urging Member States to strengthen their preparedness measures and policies. The recommended restrictions on the handling of variola virus DNA\(^1\) remained in force. Member States could introduce legislation but whether such laws would be enforceable was moot.

Other points that arose in discussion included the need for greater communication of the risk of re-emergence of a variola virus and to stratify all the risks.

With regard to public health benefits, some members of the Advisory Committee supported the continued use of live virus to evaluate antivirals against variola virus until antivirals were licenced. It was recognized that at the present time priority should be given to investigation of only the most promising compounds, rather than broad screening for new compounds.

**Vaccine development**

The Advisory Committee expressed satisfaction with progress in smallpox vaccine development and noted that in the report of its fifteenth meeting (2013)\(^2\) and after debate by the Advisory Group of Independent Experts to review the smallpox research programme (AGIES) (2013)\(^3\) it was recommended that live variola virus was no longer needed for further vaccine development. The prospect of submission of an application for licensure of an MVA-based vaccine in 2018 was encouraging.

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Research proposals for live variola virus

The Committee noted that some proposals for research with live variola virus were requests for continuations of existing projects that had been approved previously by the Advisory Committee. Timelines had been included in the proposals for the development of different antivirals being studied, and it was agreed that all future proposals from the two repositories should contain target deadlines for the conclusion of the research and that the Secretariat would modify its letters to the Collaborating Centres accordingly.

The Secretariat confirmed that it would invite submissions of time-bound proposals for both continuations of approved research projects and new projects to be undertaken in 2018. It would provide information about the new procedures, stressing that if insufficient progress were made within the timeline there will be no guarantee of an extension of the project.

Scientific Subcommittee

The Advisory Committee agreed to revise its procedures for processing proposals that were submitted. These would in future be reviewed by all members of the Committee rather than only the Scientific Subcommittee. The Secretariat would circulate proposals electronically to all members, requesting a response within two weeks (with the possibility of a one-week extension in case of unavailability). Members would base their decisions on the agreed rationale for work with live variola virus and provide specific responses in the standardized proposal review form. In a continuation of the practice of the former Subcommittee, members would be able to add text commenting on or explaining their decision. Members’ responses will be summarized by the Chairman of the Advisory Committee and the decisions relayed to the proposers. Information on the decisions would be issued in the report of the Committee’s twentieth meeting.

20. Summary

Antiviral agents

The Advisory Committee appreciated the updates on potential therapeutic agents against smallpox: tecovirimat and brincidofovir. It welcomed the likely imminent submission of an application for licensure of tecovirimat to FDA; a decision on this application was expected by the third quarter of 2018. The Advisory Committee learnt that evaluation of the activity of
brincidofovir against other double-stranded DNA viruses than variola virus was advancing, but at least 24 months’ more work was needed before an application for licensure for use against variola virus is likely.

Another therapeutic approach being examined was the use of a combination of human monoclonal antibodies that neutralize variola virus. Early results were encouraging.

Diagnostics
The Advisory Committee was pleased to learn that, in February 2017, FDA had licensed in the USA a new variola virus-specific, real-time PCR diagnostic test, and that this test was now available to certain laboratories, mostly in the USA but also in a few other countries, that were members of the US Laboratory Response Network. The test was also species-specific and might find valuable application in the field.

The Advisory Committee welcomed the progress that has been made in the development of protein-based diagnostic tests. Members of the Advisory Committee recognized the desirability of protein-based diagnostic tests for use in the field, for instance in local laboratories or as a point-of-care test, but the question remained as to whether the use of live variola virus for their further development was “essential for public health”.

The Advisory Committee noted that a diagnosis of smallpox was likely to be established on the basis of multiple lines of evidence, which include clinical assessment, electron microscopy, DNA-based PCR tests, DNA sequencing and protein-based diagnostics. A combination of tests was likely to be more powerful than individual tests.

Smallpox vaccines
The Advisory Committee further welcomed the information that an application for approval of a highly-attenuated, MVA-based smallpox vaccine was expected to be submitted in 2018 to FDA for licensure in the USA. The vaccine was already licensed as Imvamune® in Canada and as Imvanex® in the European Union.

Scientific Subcommittee
The membership of the Advisory Committee’s Scientific Subcommittee has changed over the past year. The Advisory Committee decided that, instead of using a Scientific Subcommittee,
in future the full Advisory Committee would evaluate proposals submitted by the two Collaborating Centres for further research involving the use of live variola virus. The WHO Secretariat would take over the administrative aspects of handling the receipt and distribution of proposals and review forms, ensuring prompt and timely turn-around. The proposals would be reviewed by all members of the Advisory Committee who would submit their responses within two weeks, and the Chairman would subsequently summarize the outcome.
## ANNEX 1. Research proposals submitted to WHO in 2016-2017

<table>
<thead>
<tr>
<th>Proponent and projects</th>
<th>Approved</th>
<th>Not approved</th>
<th>Majority opinion and notes</th>
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</thead>
<tbody>
<tr>
<td>VECTOR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Development of animal models to study the efficacy of therapeutic and preventive products against smallpox</td>
<td>5</td>
<td>1</td>
<td>No Significant doubts about model, with results unlikely to be accepted by regulatory authorities</td>
</tr>
<tr>
<td>CDC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Use of live variola virus to evaluate antivirals against variola virus</td>
<td>6</td>
<td>0</td>
<td>Yes</td>
</tr>
<tr>
<td>2. Use of live variola virus to determine whether mice are a suitable animal model for human smallpox</td>
<td>4</td>
<td>2</td>
<td>Yes Control experiments had been requested and permission should be given to allow completion; some doubts about time needed and correlation with human disease</td>
</tr>
<tr>
<td>3. Use of live variola virus to develop protein based diagnostic and detection assays specific for variola virus</td>
<td>4</td>
<td>1</td>
<td>Yes One comment maintained that PCR was acceptable and another raised the issue of a conflict of interest</td>
</tr>
<tr>
<td>4. Use of live variola virus to maintain and regenerate non-infectious variola derived materials for diagnostic development support</td>
<td>6</td>
<td>0</td>
<td>Yes One comment questioned the need to grow so many strains</td>
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<tr>
<td>5. Use of live variola virus to support less reactogenic vaccine development: continued evaluation of “third” generation vaccines</td>
<td>6</td>
<td>0</td>
<td>Yes Approved</td>
</tr>
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</table>
ANNEX 2. Abstracts of presentations

REPORT ON THE VARIOLA VIRUS COLLECTION AT THE WHO COLLABORATING CENTRE FOR ORTHOPOXVIRUS DIAGNOSIS AND REPOSITORY FOR VARIOLA VIRUS STRAINS AND DNA AT FBRI SRC VB VECTOR, ROSPOTREBNADZOR

R.A. Maksyutov

Federal Budgetary Research Institution - State Research Centre of Virology and Biotechnology VECTOR, Rospotrebnadzor (FBRI SRC VB VECTOR, Rospotrebnadzor), Koltsovo, Novosibirsk Region, 630559, Russian Federation

The organization of and experimentation with the Russian variola virus (VARV) collection at the WHO Collaborating Centre for Orthopoxvirus Diagnosis and Repository for Variola Virus Strains and DNA at the State Research Centre VECTOR are in compliance with national and international requirements and with WHO’s recommendations. Instructions regulating research, as well as all maintenance and control procedures, have been developed on the basis of these documents. Plans for disease control measures and response to accidents have been developed. Emergency response teams have been established for deployment in the event of accidents or emergencies.

Currently, the VARV collection comprises 120 strains, originating from the WHO African Region, South America in the Region of the Americas, South-East Asia Region, and Eastern Mediterranean.

Research using live variola virus will be continued in 2017-2018 in the following areas:

1. discovery of new antivirals for smallpox treatment and prevention
2. assessment of the neutralizing activity of vaccinees’ blood sera against live variola virus
3. development of animal models to study the efficacy of therapeutic and preventive products against smallpox..
THE WHO COLLABORATING CENTER FOR SMALLPOX AND OTHER POXVIRUSES AT THE CENTERS FOR DISEASE CONTROL AND PREVENTION ATLANTA, GA: 2017 REPORT ON THE VARIOLA COLLECTION

Victoria Olson, Christina Hutson, Ashley Kondas, Matthew Mauldin, Zachary Weiner, Yu Li, Jinxin Gao, Hui Zhao, Christine Hughes, Inger Damon

Poxvirus and Rabies Branch, Centers for Disease Control and Prevention, Atlanta, Georgia 30333, United States of America

The WHO Collaborating Center for Smallpox and other Poxviruses at the Centers for Disease Control and Prevention in Atlanta, Georgia, continues to maintain one of the two consolidated, international collections of variola virus strains. Most of these viruses were originally isolated on embryonated eggs and characterized during the final years of the intensification of the smallpox eradication campaign. The virus collection is maintained in two separate freezers, one of which is a back-up freezer which has remained largely untouched. Secure databases, which address WHO’s recommendations as well as the US Select Agent Program’s requirements have been constructed to track usage of variola virus. Annual reports on the status of these collections are provided to WHO. No new variola virus seed pools were added to the inventory between 2016 and 2017. WHO-approved research activities which have utilized variola virus from the inventory within the past year have focused on: neutralization potential of human monoclonal antibodies, evaluation of sera from vaccination regimens to evaluate efficacy based on variola virus neutralization, development of protein-based diagnostics and detection assays specific for variola virus, and determining whether mice are a suitable animal model for human smallpox.

The laboratory space was in active use from mid-July 2016 through March 2017. The laboratory underwent decontamination prior to preventative maintenance during April 2017. The laboratory was inspected by the WHO-led inspection team in May 2017. The laboratory once more became operational on 5 June, 2017. In the United States of America, variola virus is a Select Agent and subject to the Select Agents Regulations (42 CFR part 73).
USE OF LIVE VARIOLA VIRUS TO DETERMINE WHETHER MICE ARE A SUITABLE ANIMAL MODEL FOR HUMAN SMALLPOX

Christina Hutson, Ashley Kondas, Zachary Reed, Nadia Gallardo-Romero, Sharon Dietz Ostergaard, Jana Ritter, Matthew R. Mauldin, Cassandra Tansey, Clint Morgan, Johanna Salzer, Cynthia S. Goldsmith, Darin Carroll, Inger Damon, Victoria Olson

Poxvirus and Rabies Branch, Centers for Disease Control and Prevention, Atlanta, Georgia 30333, United States of America

In the United States of America, variola virus (VARV) is a Select Agent and subject to the Select Agents Regulations (42 CFR part 73). Historically, laboratory research efforts have tested several animal species for susceptibility to VARV, but as yet non-human primates (NHPs) are the only non-human animals that exhibit overt illness. However, in order to induce illness in NHPs, the required infectious dose (~1 x 10^8 VARV plaque-forming units (pfu)) is much greater than the dose required for a natural infection and the model necessitates an intravenous inoculation. Because of these limitations, several surrogate animal models of orthopoxvirus disease have been developed to evaluate the efficacy of various safer smallpox vaccines/therapeutics. Many of these models have limitations, such as short disease incubation periods, which do not resemble human smallpox. As a result, these systems are suboptimal for evaluating efficacy of antivirals as therapeutics (after onset of symptoms) and the post-exposure prophylactic use of newer smallpox vaccines. During the smallpox eradication campaign, post-exposure vaccine use was crucial in disease control and ultimate disease elimination. The discovery of a more permissive/representative VARV animal model would facilitate testing of next-generation/safer smallpox vaccines and therapeutics.

Humanized mice have become an invaluable tool for modelling human biology and disease as they provide an alternative to NHPs. Researchers are using them to investigate human-specific therapeutic candidates and evaluate the safety of biologics. Moreover, humanized mice offer a unique platform for studying human haematopoiesis, viral host-pathogen interactions and human inflammatory responses to viruses. These animals have been successfully used to investigate disease pathogenesis of multiple viruses that only infect human cells, including HIV, dengue virus and Epstein-Barr virus. These successes support the evaluation of these humanized mice as an animal model of the solely human pathogen VARV.

In 2015 under the approved research proposal, we intranasally challenged three different types of humanized female mice (BLT, hu-CD34 and PBMC, all in groups of 8) with VARV (7 x 10^3 or 7 x 10^5 pfu JAP51_hrpr strain). Mice were housed in a CDC BSL-4 laboratory as required by WHO and Select Agents Regulations. A pain score specific to mice was used to assess morbidity; if an animal scored 5 points or higher, observation was increased to twice daily. Animals that reached a score of 10 were humanely euthanized. High dose-dependent mortality was seen in the hu-CD34 and BLT mice (both dosages) beginning at day 13. The PBMC mice did not have high morbidity, and only 1 out of the 8 (in the 7 x 10^5 dose group) was euthanized before study end on account of its pain score. Large loads of viable virus were consistently harvested in most titrated tissues, regardless of mouse strain/challenge dose. The findings from the 2015 study suggested that humanized mice (particularly BLT and hu-CD34) have the potential to serve as a model for smallpox. Our preliminary study identified that all three mouse strains were susceptible to VARV, and subsequent immunohistochemical and pathological specimen analyses strongly support infection as the cause of morbidity in mice that succumbed to disease. Of animals intranasally challenged with 7 x 10^3 or 7 x 10^5 pfu 100%, 88%, or 13% manifested signs of morbidity (BLT, hu-CD34 and PBMC mice, respectively) necessitating euthanasia, with an incubation period of 13-21 days post-infection. The features of this animal model make it suitable for efficacy testing of potential antiviral therapeutics against VARV infection, namely the ability of therapeutics to protect mice from a VARV challenge, and limit spread of virus from the inoculation site.
Our initial study minimized animal numbers in order to ascertain the susceptibility of multiple humanized mouse strains to VARV. Based on those results, additional studies were warranted to determine whether the study observations were due to the presence of the introduced components of a human immune system or a feature of the background NOD scid gamma (NSG) mouse. In 2017, we evaluated whether immunosuppressed NSG mice can support spread of VARV. As with the initial evaluation of humanized mice, we intranasally challenged NSG mice with VARV in different doses ($5 \times 10^4$ or $4 \times 10^6$ pfu JAP51_hrpr strain). Three humanized NSG mice (PBMCs) were used as positive controls and were challenged with the high dose inoculum. Mice were housed in a CDC BSL-4 laboratory and similar sampling and observation were done during this follow-up study. Clinical signs and weight loss were minimal or absent in the NSG mice (both inoculums) and all NSG animals were still alive at study end (21 days post-infection). As in the initial study, PBMC hu-mice began displaying clinical signs late in the study (about day 19) and one animal had to be euthanized on day 19 due to a pain score of 10. Complete necropsies were performed and molecular analysis is underway. Preliminary results of study observations and tissue analyses will be presented at the meeting.
USE OF LIVE VARIOLA VIRUS TO MAINTAIN AND REGENERATE NON-INFECTIONOUS VARIOLA DERIVED MATERIALS FOR DIAGNOSTIC DEVELOPMENT SUPPORT

Victoria Olson, Ashley Kondas, Kimberly Wilkins, Christina Hutson, Inger Damon, Yu Li

Poxvirus and Rabies Branch, Centers for Disease Control and Prevention, Atlanta, Georgia 30333, United States of America

The ability to validate nucleic acid-based and protein-based diagnostic assays is crucial for early detection and recognition of smallpox should a bioterror incident result in reintroduction of variola virus. The consequences of either false-negative or false-positive results will significantly impact global public health. “Older” nucleic acid diagnostic platforms (from 1990 to 2000) are no longer being supported by some companies, necessitating development of newer platforms that must be reviewed by the US Food and Drug Administration. As has occurred in the recent past, new isolates of orthopoxvirus are being identified and can confound current diagnostic assays. The maintenance of variola virus DNA and antigen stocks at the WHO Collaborating Center for Smallpox and other Poxvirus Infections remains important for future diagnostic development and validation. In the United States of America, variola virus is a Select Agent and subject to the Select Agents Regulations (42 CFR part 73). Building rapid and accurate diagnostic capacity for use in laboratories world-wide is critical for successful containment of disease in the event of a re-emergence of smallpox. Evidence of this occurred during the response to the 2014-2015 Ebola virus disease outbreak in West Africa, where there was a crucial need for rapid and accurate diagnostic capacity in remote or central laboratories for effective disease control. The presentation will update the results from use of non-infectious variola virus to validate several DNA diagnostic assays.

Assay validation is substantially more robust when tested with extracted genomic DNA, representative of what would be extracted from a clinical isolate, rather than plasmids expressing the target portions of DNA as internal assay positive controls. For sensitivity analyses, use of virus DNA that has been extracted from purified virions allows a calculation of the limit of detection. Such materials will continue to be used to validate detection assays, as well as human clinical diagnostics, developed by WHO’s Member States. Over the past year we have focused on gaining regulatory approval of assays as well as validation of new variola virus-specific assays, both internally at CDC and with an external collaborator, Altona Diagnostics. Additionally, we have optimized the combination of two variola virus-specific real-time PCR assays and one orthopoxvirus generic PCR assay in a multiplex format that can be used in a point-of-care diagnostic platform. We are continuing the optimization process to include a fourth PCR assay for sample integrity to improve the diagnostic algorithm.

Additional external collaborators: Kaya Kreuzfeld, Stephan Olschlager, Altona Diagnostics.
USE OF LIVE VARIOLA VIRUS TO EVALUATE MONOCLONAL ANTIVIRALS AGAINST VARIOLA VIRUS

Ashley Kondas, Christina Hutson, Inger Damon, Victoria Olson

Poxvirus and Rabies Branch, Centers for Disease Control and Prevention, Atlanta, Georgia 30333, United States of America

The primary objective of preparedness against smallpox bioterrorism is to save lives if smallpox re-emerges. In the United States of America, variola virus is a Select Agent and is subject to the Select Agents Regulations (42 CFR part 73). The development of antiviral strategies may be important in outbreak response efforts as well as in disease treatment. Current considerations have suggested the need for two antiviral compounds, with discrete mechanisms of action, to be licensed and available for use. Some historical literature that suggests that treatment with immune products from persons who have convalesced from smallpox may provide protection against smallpox. To date, there are no antiviral compounds or monoclonal antibodies (mAbs) approved by the Food and Drug Administration (FDA) for treatment of poxvirus infections. Vaccinia Immune Globulin (VIG) is licensed by FDA for treatment of complications from vaccination with vaccinia virus. However, the supply of VIG is limited since it is no longer mass produced after the eradication of smallpox.

Recent efforts (in collaboration with the Vanderbilt Vaccine Center) to identify antiviral therapeutics against variola virus have focused on the use of mAbs. Using a highly-optimized human hybridoma technology, the external collaborators created a panel of mAbs from people immunized with vaccinia virus or people who survived monkeypox virus or variola virus infection. In vitro characterization identified 48 mAbs directed against multiple viral proteins capable of complement-dependent neutralization of the intracellular mature virion or external enveloped virion form of an orthopoxvirus. Two different combinations of mAbs (Mix4 and Mix6) were designed that had high capacity to neutralize both forms of the virus across multiple species. When Mix6 was used as a therapy it provided complete protection against a lethal respiratory challenge with vaccinia virus in mice. Previously at CDC, we demonstrated that both mAb combinations efficiently neutralized the intracellular mature virion of variola virus better than VIG. Commercial entities have also begun production of mAbs directed against orthopoxviruses. Two human antibodies that target H3 and B5 viral proteins have been designed and characterized in vivo. In the progressive vaccinia mouse model, pre-treatment of these mAbs individually with vaccinia virus increased survival time and the mice maintained weight. Pre-treatment combination therapy, however, provides significantly better protection against disease and death than treatment with individual mAbs or VIG. Furthermore, treatment with combined mAbs has shown post-exposure efficacy in both SCID/vaccinia virus and rabbit/rabbitpox virus models. Another commercial entity has developed a mAb cocktail targeting L1 and A33 viral proteins. Single administration of this mAb cocktail provides post-exposure protection in various mouse models with several orthopoxviruses. In the BALB/c vaccinia virus model, the mAb cocktail demonstrated greater capacity to neutralize the virus than VIG. In a non-human primate monkeypox virus challenge study, cocktail treatment given in a single dose post-exposure all animals survived, there were fewer pox lesions and reduced viraemia compared to VIG or vehicle alone. The in vivo data are promising yet the ability of these mAbs to neutralize variola virus is still unknown. Therefore, it is important to demonstrate the ability of these mAb cocktails to neutralize variola virus in our surrogate in vitro assay: the plaque-reduction neutralization assay.

1 Additional external collaborators: Iuliia Gilchuk, James Crowe, Jr.
The Vanderbilt Vaccine Center, Vanderbilt University Medical Center, Nashville, TN 37212, United States of America
The presentation will provide an update on progress in determining the capability of the Vanderbilt Vaccine Center mAbs to neutralize variola virus in vitro using our recently optimized plaque-reduction neutralization assay as well as our initial stages of efficacy studies with these mAbs within the monkeypox virus/prairie dog model. Additionally we shall provide more detail on the data regarding efficacy of commercially produced mAbs to neutralize orthopoxviruses.
USE OF LIVE VARIOLA VIRUS TO DEVELOP VIRUS SPECIFIC PROTEIN-BASED DIAGNOSTIC AND DETECTION ASSAYS

Victoria Olson, Michael Townsend, Ashley Kondas, Christina Hutson, Nadia Gallardo, Inger Damon, Subbian S. Panayampalli

Poxvirus and Rabies Branch, Centers for Disease Control and Prevention, Atlanta, Georgia 30333, United States of America

The ability to validate protein-based diagnostic assays is crucial for the WHO Collaborating Center to detect and recognize smallpox early should a bioterror incident result in reintroduction of variola virus. The consequences of either false-negative or false-positive results will significantly impact global public health. In the United States of America, variola virus (VARV) is a Select Agent and is subject to the Select Agents Regulations (42 CFR part 73). During the response to the 2014-2015 Ebola virus disease outbreak in West Africa, the need for rapid and accurate diagnostic capacity in remote or central laboratories was critical for successful disease containment.

Since late 2011, studies have continued on the characterization of monoclonal antibody (mAbs), viral antigen-capture assays, and protein microarray development applicable to variola virus (VARV). Because advanced diagnostics require expensive assay platforms and infrastructure, we have initially focused on simpler assays that would be amenable for use in resource-constrained areas. Antibodies that could be used to detect VARV specifically have been assessed using virus-capture ELISA and lateral flow assays. Promising initial sensitivity assessments to γ-irradiated VARV of 1 x 10^4 pfu/ml by ELISA and 1.1 x 10^5 by lateral flow assays have been found. Efforts have also focused on assessing specificity to VARV and validating that mAbs bind both live and γ-irradiated virus. Results have demonstrated differential binding to live compared with γ-irradiated VARV depending on which mAb is used. We continue to use a VARV-encoded protein microarray to describe antibody responses against individual VARV proteins. These results are aiding our understanding of humoral immunity to orthopoxviruses and facilitating differentiation of vaccination from orthopoxvirus infection. The virus capture and microarray assay updates will be presented at the meeting.

USE OF LIVE VARIOLA VIRUS TO SUPPORT LESS-REACTOGENIC VACCINE DEVELOPMENT: CONTINUED EVALUATION OF THIRD-GENERATION VACCINES

Christina Hutson, Matthew Mauldin, Ashley Kondas, Christine Hughes, Whitni Davidson, Inger Damon, Victoria Olson

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Poxvirus and Rabies Branch, Centers for Disease Control and Prevention, Atlanta, Georgia 30333, United States of America

Variola virus neutralization in vitro remains an informative surrogate measure of smallpox vaccine efficacy. Our prior studies, using sera from people vaccinated with vaccinia virus (MVA, Dryvax or Lc16m8) have indicated that neutralization endpoint titres may differ when using different target viruses. Slight differences in orthopoxvirus antigens are likely to account for these differences. Furthermore, our data demonstrated that vaccinees’ sera neutralized different strains of variola virus with different levels of efficiency. The plaque-reduction neutralization test (PRNT), which measures the ability of immune sera to neutralize mature virus forms (MV), has been used as a primary endpoint for the evaluation of vaccines. However, the ability of a vaccinee’s serum to neutralize the extracellular enveloped virus (EEV) form of virus may be critical for vaccine efficacy since EEV is important for viral dissemination and ultimate disease pathogenesis.

1 Additional external collaborator: Darja Schmidt, Bavarian Nordic.
The development of new vaccines has included significant focus on the use of attenuated vaccine strains, such as modified vaccinia Ankara (MVA), and Lc16m8. These “third-generation” vaccines, however, were never tested directly for efficacy against smallpox during the eradication campaign, as most were developed towards the end of that era or after eradication. We have found a statistically significant difference in neutralization titres of Lc16m8 vaccinees’ sera when using different target viruses (variola virus - heterologous target versus vaccinia virus - homologous target). Therefore, evaluation of the ability of sera, generated through animal or human trials with less-reactogenic smallpox vaccines, to neutralize MV and EEV forms of variola virus will provide a more informative surrogate measure of efficacy. The role of variola virus neutralization as a surrogate marker for vaccine efficacy is particularly valuable for the evaluation of vaccines like MVA that do not elicit a “take”, the traditional measure of vaccine success.

We have optimized methodologies for determining MV-neutralizing capacity of sera from people vaccinated with Imvamune® (produced by Bavarian Nordic) against variola virus (VARV) to standardize, remove subjectivity, and increase throughput of the assay. This presentation will update changes in the PRNT assay developed at the CDC Poxvirus and Rabies Branch to increase similarity to that conducted by Bavarian Nordic. As CDC’s standard operating procedures for the VARV PRNT assay were altered, parameters of the assay were re-evaluated. Once the standard operating procedures were finalized, we determined variability between days and between operators, as well as location within the plate. Through this initial evaluation, we found good reproducibility in VARV PRNT results between days and operators using the newly optimized assay incorporating a 12-well cell culture plate, regardless of the location within the plate. Following this initial evaluation, we further evaluated the ability of the VARV PRNT assay to distinguish between different “neutralization groups” of serum. This control serum, obtained from Bavarian Nordic, derived from groups of individuals who had previously been found to have varying levels of neutralization efficacy (negative, very low, low, medium and high neutralization against vaccinia virus). The results from these two sets of assay analyses (VARV PRNT parameter and VARV PRNT neutralization sensitivity evaluations) are currently being analysed and results will be shared with Food and Drug Administration. These data are essential for future submissions towards regulatory approval for these “third”-generation vaccines. In the United States, variola virus is a Select Agent and is subject to the Select Agents Regulations (42 CFR part 73).
PRECLINICAL STUDIES ON A CANDIDATE SMALLPOX VACCINE, VACDELTA6

S.N. Shchelkunov, S.N. Yakubitsky, A.E. Nesterov, A.V. Zaykovskaya, A.A. Sergeev, I.V. Kolosova, E.V. Gavrilova, R.A. Maksyutov

Federal Budgetary Research Institution - State Research Center of Virology and Biotechnology VECTOR, Rospotrebnadzor (FBRI SRC VB VECTOR, Rospotrebnadzor), Koltsovo, Novosibirsk Region, 630559, Russian Federation

Attenuated fourth-generation smallpox vaccines are designed by introducing genetically engineered deletions and/or insertions leading to alterations in the genes of vaccinia virus (VACV) that inter alia control the body's defence reactions against viral infection and determine the range of susceptible hosts for this virus. Based on the vaccinia virus strain LIVP that is used for human vaccinations in the Russian Federation, we have designed a recombinant variant of the virus, VACΔ6, with directed alteration of genes encoding γ interferon-binding protein (B8R), complement-binding protein (C3L), haemagglutinin (A56R), thymidine kinase (J2R), the Bcl2-like inhibitor of apoptosis (N1L), and the A35R gene that controls antigen presentation by major histocompatibility complex (class II) molecules.

An efficacy study of LIVP and VACΔ6 in cultures of CV-1, Vero and 4647 cells has not revealed any significant differences in this parameter between the viruses compared.

Three batches of a candidate vaccine against smallpox based on VACΔ6 have been produced and preclinical studies on the specific activity of this vaccine in vitro and in vivo have been conducted.
RETROSPECTIVE GENETIC ANALYSIS OF GENOMES OF VARIOLA VIRUS STRAINS

E.V. Gavrilova, T.V. Tregubchak, A.N. Shvalov, S.N. Shchelkunov, R.A. Maksyutov

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VECTOR, Rospotrebnadzor (FBRI SRC VB VECTOR, Rospotrebnadzor), Koltsovo,
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We have conducted retrospective genetic analysis of the outbreak of smallpox in Moscow in 1959-1960. Complete sequences of the genomes of four variola virus (VARV) strains with known virus transmission chains were determined using Illumina-based Next-Generation Sequencing technology: two first-generation strains, M-N-60 and M-Sur-60, one second-generation strain, M-Bl-60, and one third-generation strain, M-A-60. A comparative analysis of the genome sequences showed that all four of these VARV strains within all the three generations did not practically differ. Minor differences between genomic DNA of these strains consist in the number of AT repeats in the intergenic region 164678-164703 (in relation to the nucleotide sequence of the M-N-60 genome).

Each of the four VARV strains examined included a mixture of virus variants with varying numbers of AT repeats: M-Sur-60 (10-12), M-N-60 (11-15), M-Bl-60 (10-13), M-A-60 (11-13). The data obtained demonstrate a high stability of VARV during transmission within the human population over several generations within a single outbreak.

Also as part of the retrospective genetic analysis, complete genome sequences of 20 VARV strains were identified (including four clinical specimens with no passage history: strains Brazil 128, India 164, Nepal 89 and Indon-9) that had been isolated in 1952-1975 in different geographical regions of the world and maintained in the collection at VECTOR, as the WHO Collaborating Center for Orthopoxvirus Diagnosis and Repository for Variola Virus Strains and DNA.

Phylogenetic analysis of 24 VARV strains, sequenced as part of this research effort and compared with all available full-length coding genome sequences of 49 VARV strains, previously sequenced and uploaded to GenBank, showed the strains under study to cluster predominantly according to their geographical origin and the year of outbreak and in accordance with previously sequenced genomes.
UPDATE ON THE NON-REPLICATING SMALLPOX VACCINE IMVANEX®
(IMVAMUNE®)

Nathaly Samy

Senior Vice President Clinical Development, Bavarian Nordic, D-82152 Martinsried, Germany

The recent de novo synthesis of horsepoxvirus could potentially lead to the widespread synthesis of other orthopoxviruses, such as variola virus. The reintroduction of smallpox into the human population is thus a real possibility. Furthermore, the current spread of related zoonotic orthopoxviruses, such as monkeypox, cowpox and vaccinia virus, has raised significant concerns among public health officials.

Traditional replicating smallpox vaccines are associated with serious adverse events, including an increased risk of myo-/peri-carditis. A mass vaccination programme with these vaccines would be associated with severe risks for immunocompromised subjects, pregnant women and individuals with exfoliating skin diseases. Safer strategies are therefore needed to protect against this latent threat. The non-replicating and highly-attenuated modified vaccinia Ankara (MVA-BN®, trade name IMVAMUNE® outside the European Union, invented name IMVANEX® in the European Union) has undergone extensive research with more than 8800 vaccinated subjects in completed and ongoing clinical trials, including healthy and at-risk populations such as subjects infected with HIV and individuals with atopic dermatitis. Additional programmes are investigating MVA-based recombinant agents against different indications, comprising more than 2500 subjects in completed and ongoing clinical trials. The entire safety database of subjects receiving IMVAMUNE and IMVAMUNE-based products encompasses data on more than 10 500 individuals. IMVAMUNE has been shown to be safe and well-tolerated in all subjects, without any signs of cardiac toxicity in healthy individuals and populations previously contraindicated to receive replicating smallpox vaccines. This outstanding safety profile distinguishes IMVAMUNE from traditional replicating smallpox vaccines. In 2010, IMVAMUNE was granted a pre-emergency use authorization status in the USA for vaccination of HIV-infected individuals and subjects with atopic dermatitis. IMVAMUNE has been licensed in the European Union and Canada since 2013.

The potential of IMVAMUNE to protect against other orthopoxviruses is under investigation in an ongoing trial performed by CDC, evaluating IMVAMUNE in conditions of natural orthopoxvirus transmission. A total of 1002 health care workers at risk of exposure to monkeypoxvirus were enrolled in the Democratic Republic of the Congo and completed the day 42 follow-up visit (>97% retention rate). No serious adverse event has been reported.

An ongoing phase III non-inferiority trial compares indicators of efficacy of IMVAMUNE to those of the replicating smallpox vaccine ACAM2000. Immunogenicity is assessed through vaccinia-specific PRNT assay. The pioneering methods agreed by FDA to assess the attenuation of the “take” (by vaccination with IMVAMUNE before administration of ACAM2000) include a group-wise comparison of lesion areas using the maximum of two measurements per subject, and calculations of a lesion area attenuation ratio.

The robust safety and immunogenicity profile together with the ongoing analysis of data collected in the aforementioned trials provide the necessary evidence to include IMVAMUNE in pre- and post-event vaccination programmes to protect against smallpox and related orthopoxvirus infections.
PROGRESS TOWARDS REGULATORY APPROVAL OF TPOXX® FOR THE TREATMENT OF SMALLPOX AND OTHER ORTHOPOXVIRUS INFECTIONS IN HUMANS

Dennis E. Hruby

SIGA Technologies, Inc., Suite 110, 4575 SW Research Way, Corvallis, Oregon 97333, United States of America

To counter the threat of smallpox as a bioterror weapon, SIGA Technologies is developing tecovirimat (TPOXX®, ST-246) for the treatment of smallpox and other human orthopoxvirus infections. Both oral and intravenous formulations of tecovirimat are in advanced development. For the oral formulation, all the necessary studies have been completed and the New Drug Application to FDA is currently in preparation. The application is being provided to FDA as a rolling submission with the final modules expected to be filed by the end of 2017. If the application is satisfactory, it is hoped that regulatory approval will be granted in the third quarter of 2018. The company has completed delivery of the initial order of two million courses of tecovirimat to the US Strategic National Stockpile. For the intravenous formulation of tecovirimat, the company has completed a single ascending-dose clinical trial. No serious adverse event was noted, and dose linearity was observed, thus enabling an appropriate intravenous dose to be selected to approximate the pharmacokinetic parameters measured following oral tecovirimat dosing. Following recent FDA guidance, the company is moving forward with a bioavailability/multiple-dose study to advance this programme towards a subsequent regulatory filing for the intravenous tecovirimat product.
UPDATE ON THE DEVELOPMENT OF BRINCIDOFOVIR FOR SMALLPOX

D. Fraser Gray

Chimerix Inc, Durham, North Carolina 27713, United States of America

Brincidofovir (BCV) is a potent in vitro inhibitor of multiple dsDNA viruses including adenovirus, cytomegalovirus and variola virus (VARV). Following intracellular metabolism of BCV to cidofovir-diphosphate), cidofovir is incorporated by the viral polymerase into nascent-chain viral DNA, inhibiting viral replication. Generation of resistance in orthopoxviruses in vitro is typically slow (taking months) and requires multiple mutations for high-level resistance, and resistant strains have impaired growth, all of which suggests a high barrier to resistance to BCV. Given the unique mechanism of action, BCV can be used in combination with other smallpox antivirals. BCV has been administered to more than 1400 patients in our cytomegalovirus, adenovirus, open-label and named patient/emergency investigational new drug programmes, including immunocompromised patients, those with renal and/or hepatic impairment, and paediatric subjects as young as one month of age. BCV is available in tablet and liquid formulations, with an intravenous formulation in clinical development; manufacturing has been validated at commercial scale. BCV has demonstrated efficacy in several studies using both intradermal rabbitpox and mouse ectromelia virus models. Specifically, in a pivotal rabbitpox efficacy study, animals received either placebo or BCV regimen consisting of an initial 20 mg/kg orally, followed by two 5 mg/kg doses at 48-hour intervals. BCV demonstrated 100% survival with treatment initiated at onset of fever, the first clinical sign of confirmed infection. Compared to the placebo mortality rate of 53%, a statistically significant reduction in mortality was observed when BCV was administered immediately, or at 24 or 48 hours after the onset of fever ($P<0.05$ vs placebo, Fisher’s exact test). Further, BCV treatment was associated with a reduction in circulating infectious virus in infected rabbits, with earlier BCV intervention associated with a more profound reduction. The company is conducting final studies on the ectromelia/mouse model and expects to complete the pivotal efficacy study with BCV in 2018. BCV will likely be dosed at 200 mg/week for three weeks for use against smallpox. At this dose and duration, BCV has demonstrated acceptable safety and tolerability in healthy subjects and in immunocompromised patients for whom a live vaccine is contraindicated.
FDA PERSPECTIVE ON THE DEVELOPMENT AND APPROVAL OF SMALLPOX MEDICAL COUNTERMEASURES

Edward Cox

Director, Office of Antimicrobial Products, Center for Drug Evaluation and Research, US Food and Drug Administration, Silver Spring, Maryland 20993, United States of America

The Food and Drug Administration (FDA) is responsible for ensuring the safety, effectiveness and security of medical products, including medical countermeasures. FDA also works to help to foster the development of medical countermeasures—with the goal of achieving FDA approval— as well as facilitating timely access to medical countermeasures in the event of a public health emergency through an appropriate regulatory mechanism.

The US Government is supporting the development of smallpox medical countermeasures, including medicines, vaccines and diagnostic tests. The presentation highlights the regulatory progress made since the Advisory Committee’s 18th meeting as well as some of the continuing challenges.

Smallpox medical countermeasures present unique and complex scientific challenges because the evidence upon which regulatory decisions are based is challenging to obtain owing to the fact that there is no smallpox disease in the world and animal models that adequately represent smallpox disease are not available.

Working closely with developers of smallpox medical countermeasures, FDA has established feasible and appropriate regulatory pathways for their approval. The focus of FDA’s interactions with those developers is on providing feedback on proposed studies to support clinical safety, pharmacokinetic and animal model efficacy studies for antiviral agents, pivotal efficacy studies and bridging studies for attenuated vaccines, and requirements for the validation of diagnostic tests. In 2015, FDA issued final guidance on developing products under the Animal Rule. The guidance enhances and clarifies FDA’s expectations and recommendations on developing products under the Animal Rule based on comments received to previous draft guidance and the aggregate experience to date in developing products under the Animal Rule.

1 The term “approval” refers to “FDA-approval, licensure, or clearance” under sections 505, 510(k), or 515 of the Federal Food, Drug, and Cosmetic Act or section 351 of the Public Health Service Act.
ANNEX 3. Provisional agenda

DAY ONE - 1 November 2017

09:00 – 09:30 Members and Advisors only – closed session

09:30 – 9:45 OPENING – Dr Peter Salama, Executive Director, WHO Health Emergencies

Welcome remarks – Dr Sylvie Briand, Director, Department of Infectious Hazard Management, WHE

VARIOLA VIRUS REPORTS

09:45 – 10:00 Report of the Secretariat – WHO Smallpox Secretariat – A. Khalakdina

10:00 – 10:15 Update on research proposals submitted to WHO in 2017 – Subcommittee

10:15 – 10:30 Update on Smallpox Vaccine Emergency Stockpile and Smallpox Operational Framework – T. Nguyen

10:30 – 11:00 TEA/COFFEE BREAK

11:00 – 11:20 Report on the variola virus collection at the WHO Collaborating Center Repository in VECTOR, Koltsovo, Novosibirsk Region, Russian Federation – R. Maksyutov

11:20 – 11:40 Report on the variola virus collection at the WHO Collaborating Center for Smallpox and other Poxviruses at the Centers for Disease Control and Prevention, Atlanta, Georgia, USA – I. Damon

VARIOLA VIRUS RESEARCH UPDATE – 2017

ANIMAL MODELS

11:40 – 12:00 Use of live variola virus to determine whether mice are a suitable animal model for human smallpox – C. Hutson

DIAGNOSTICS

12:00 – 12:20 Use of live variola virus to maintain and regenerate non-infectious variola derived materials for diagnostic development support – V. Olson

12:20 – 13:20 LUNCH

13:20 – 13:50 Use of live variola virus to develop protein based diagnostic and detection assays specific for variola virus – V. Olson

ANTIVIRALS

13:50 – 14:10 Use of live variola virus to evaluate antiviral agents against smallpox – V. Olson

VACCINES
14:10 – 14:30  Use of live variola virus to support less-reactogenic vaccine development: continued evaluation of “third” generation vaccines – C. Hutson

**OTHER SMALLPOX RESEARCH**
14:30 – 14:45  Preclinical studies on a candidate smallpox vaccine, VACΔ6 – S.N. Shchelkunov
14:45 – 15:00  Retrospective genetic analysis of genomes of variola virus strains – E.V. Gavrilova
15:00 – 15:30  TEA/COFFEE BREAK

**LICENSED UPDATES**
15:30 – 15:45  Update on smallpox vaccine IMVANEX® (IMVAMUNE®) – N. Samy
15:45 – 16:00  Progress towards approval and deployment of Arestvyr® (ST-246) – D. Hruby
16:00 – 16:15  Update on the development of brincidofovir (CMX001) for smallpox – F. Gray
16:15 – 16:30  FDA perspective on the development and approval of smallpox medical countermeasures – E. Cox
16:30 – 17:30  GENERAL DISCUSSION

CLOSE OF DAY ONE

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**DAY TWO - 2 November 2017**

**Closed discussion for Members and Advisors of the Advisory Committee on Variola Virus Research**

09:00 – 09:30  Update variola virus repositories biosafety inspection visits – K. Kojima
09:30 – 10.30  Preparedness measures for smallpox and other dangerous pathogens
10:30 – 11:00  TEA/COFFEE BREAK
11:00 – 12:30  Discussion on future orthopoxvirus research priorities
12:30 – 13:30  LUNCH

**Closed final discussion for Members of the Advisory Committee on Variola Virus Research**

13:30 – 15:00  Finalization of draft recommendations of the Advisory Committee on Variola Virus Research

CLOSE OF MEETING
ANNEX 4. List of participants

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Mr David FitzSimons, Rapporteur